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ACROPETAL TRANSLOCATION OF ACEPHATE INTO TERMINAL SHOOTS OF JEFFREY PINE FOR THE CHEMICAL CONTROL OF WESTERN PINE SHOOT BORER

by
C. E. Crisp, T. W. Koerber, C. E. Richmond
Pacific Southwest Forest and Range Experiment Station
United States Department of Agriculture, Forest Service
1960 Addison Ave., Berkeley, CA 94701
and
B. H. Roettgering
Pacific Southwest Region
United States Department of Agriculture, Forest Service
San Francisco, CA

ABSTRACT

The distribution, concentration and persistence of acephate insecticide (O,S-dimethyl acetylphosphoramidothioate) (Orthene®) was determined in the protected terminal shoots (sinks) of 15-year-old Jeffrey pine (*Pinus jeffreyi* L.) and related to efficacy for control of the western pine shoot borer (*Eucosma sonomana* Kerf.). Acephate was applied at 2.4 g/ℓ to the previous years growth (source). The concentration of acephate and its metabolite, methamidophos, in the tissues of terminal shoots at 30 days post-treatment was 3.27 ppm, distributed as follows: needles, 8.50 ppm; bark, 1.10 ppm; xylem, 0.32 ppm; and pith, 1.44 ppm. Acephate residue concentrations were evenly distributed at 1-cm intervals from the base to the tip of the terminal shoot. Lateral shoots at the base of the leader were used to determine the persistence of acephate. The results showed the following post-spray levels: 2 days, 2.32 ppm; 7 days, 2.91 ppm; 14 days, 3.18 ppm; and 21 days, 0.77 ppm. Under the conditions of this test, acephate was not effective against larvae of the western pine shoot borer, because the acephate was translocated and concentrated in the new needles rather than in the pith of the terminal shoot where the larvae feed.

INTRODUCTION[1/,2/,3/]

Shoot borers and tip moths feeding on the terminal shoots of conifers reduce the rate of height growth. For example, studies on *E. sonomana* show that in plantations where terminal shoots are repeatedly infested by the western pine shoot borer (greater than 60 percent of terminals yearly) the cumulative growth loss may be enough to cause a 20 to 22 percent reduction in stem volume (Stoszek 1973).[4/] Stoszek's estimate is conservative, since value reduction due to forking and stem deformation caused by loss of leader dominance was not considered. Effective economical control measures are needed to suppress shoot-and-tip moth populations.

The primary tissue eaten is pith from the current year's growth. Some vascular tissue is destroyed in this process. The peak feeding period occurs when the maximum quantity of photosynthate is being translocated from the photosynthate source (the old needles adjacent to and on the leader shoot) to the photosnythate sink (the developing bud and leader for the current year).

The primary objectives of this study were to determine the following:

- Effectiveness of acephate, a phloem-mobile systemic insecticide, for preventing damage by the western pine shoot borer
- Distribution, concentration and persistence of the insecticide in pine trees
- Effectiveness of the phloem transport mechanism in moving acephate from treated foliage to untreated new shoots.

REVIEW OF LITERATURE

Examples of insects in the shoot-and-tip moth category are *Dioryctria* spp., *Rhyacionia* spp., *Petrova* spp., *Eucosma* spp., and *Laspeyresia* spp. The damaged caused by these insects seldom kills the tree, but can cause serious stunting and deformation. The result is a very bushy tree of poor commercial quality. Remedial chemotherapy in response to such infestation is dependent on synchronizing the translocation of a toxicant to the insect feeding site with the start of feeding. Here success requires an understanding of the mechanism and rate of absorption, translocation, and metabolism of toxicants so that, before insect feeding starts, treatments will produce lethal concentrations of insecticide in the food supply.

Larvae of *E. sonomana* feed in the pith of terminal and upper whorl lateral shoots. Peak mining period occurs shortly after bud break (Wilson 1972). Stoszek (1973) reports that hatching of larvae occurs at the time of bud elongation. The

first instar larvae bore through the bud scale base into the pith and feed through the pith downward through the shoot axis often reversing direction. As the larvae proceed downward, a corresponding retardation of shoot and needle length occurs.

Past and current chemical strategies for control of shoot-and-tip feeding insects have used contact and xylem-mobile systemic insecticides. Dimethoate and malathion are currently registered (U.S. Department of Agriculture 1974) for control of insects with similar feeding habits (Haverbeke and others 1971), but no effective treatment is known for *E. sonomana*. Dimethoate and malathion are xylem-mobile (Crisp 1972) but, regardless of the method of application, will not phloem-transport to the leader. Thus, contact insecticides may be toxic topically to the larvae but are of little value once feeding in the pith is initiated.

Larson (1976) and Werner (1974) evaluated systemic activity of acephate and determined it to be very xylem-mobile; however, the methods used did not permit a clear interpretation about phloem-mobility. Bull (1979) reported that within cotton plants, acephate or its metabolites are translocated to all parts of the plant, including the rapidly growing fruiting bodies which are excellent photosynthate sinks.

LaBerge (1974) and Brown and Eads (1975) reported that foliar application of acephate provides excellent control of the Nantucket pine tip moth. Because acephate shows some phloem mobility, it has received considerable interest (Richmond and others 1979, Bull 1979, Hall and others 1978, Brewer and O'Neal 1977, Sundaram and others 1977, Sundaram and Hopewell 1976, and Larson 1976). Based on this information, a chemotherapeutic approach using insecticides that exhibit some phloem mobility would seem to have important advantages over present methods.

If long distance phloem transport is to be used to collect, distribute, and concentrate systemic insecticides to kill larvae feeding on the tissues of shoots and leaders, a thorough knowledge of the host tree's phloem physiology would be helpful. Phloem transport in plants has been reviewed by Crafts and Crisp (1971). The sieve cells of gymnosperms are living conduits for the translocation of photosynthates, and acropetal transport through them normally takes place in spring. Phloem-loading of foreign molecules at the source is critical in the translocation sequence, since the foreign molecules must be absorbed into the assimilate stream (Crisp and Look 1979). Demand at the sink and supply at the source regulate translocation (Zimmermann and Brown 1971). Translocation is always from source to sink. Flow will continue towards the sink so long as there is a turgor differential.

An effective phloem-mobile systemic insecticide should exhibit some unique characteristics: water solubility, a molecular structure with a carboxyl group or a group that mimics it (for example, phosphoramido), resistance to biodegradation in the host, and phloem loading in concentrations sufficient to make the sink toxic to feeding insects (Crisp and Look 1979). Acephate and its toxic metabolite, methamidophos, have all of these characteristics; however, some of the characteristics are untested in Jeffrey pine.

METHODS AND MATERIALS

Experimental Design

Field testing was based on a completely randomized experiment. Variables measured were efficacy, residue levels in different tissues at different times, spray deposit, and leader growth. Treatments were check and acephate. Each treatment consisted of 100 replications (each tree was considered a replication). Ten trees were selected at random for spray deposit assessment within each treatment.

Selection of Study Trees

The study area was a plantation located 6 kilometers northwest of Hobart Mills, California, at an elevation of 1,900 meters. The trees were 15 years old and are growing on 6 to 20 percent slopes with a northeast aspect. The plantation was thinned in 1963 and sprayed with 2,4-D to reduce brush competition. The soil is of the Fugawee soil class and is lightly to strongly acidic. Evidently, *E. sonomana* has persistently infested these trees for some years. The trees were all Jeffrey pine and had an average height of 1.5 meters. Average annual leader growth of all trees in the plantation for the years 1968 through 1974 was approximately 38 centimeters.

Formulation of Acephate and Time of Application

Acephate (Orthene^{R} 75S) was formulated at 2.4 g/ℓ of water immediately prior to use. To assess spray deposits, the dye Rhodamine B Extra S was added at the rate of 1 g/ℓ.

The acephate solution was applied as a coarse spray from an 11.4-liter Hudson sprayer pressurized to 3.52 kg/cm^2 by connection to a tank of compressed air. The delivery rate of the sprayer was determined and the spray application timed with a stop watch so as to spray over each tree for the time required to deliver 0.94 liters of spray. The trees were treated on June 26, at which time the new terminal shoots of the trees had elongated to an average length of 7.62 centimeters. The adult flight

and oviposition period of *E. sonomana* started about 30 days prior to the spray application. The majority of the eggs had hatched and first and second instar larvae were feeding inside the new shoots.

The new terminal and top whorl of lateral shoots of each tree were enclosed in a polyethylene bag immediately prior to spray application to prevent contact action or direct absorption of the insecticide. This precaution was taken to ensure that the insecticide later detected in sample shoots was due to absorption and phloem transport from the previous year's foliage. The bags were removed as soon as the spray stopped dripping to avoid overheating the shoots.

Sample branches were removed from 19 randomly chosen trees after the spray deposit dried. Each sample branch was placed in a polyethylene bag, tagged to identify the tree and returned to the laboratory for deposit assessment using the method of Maksymiuk and others (1971). The dye was removed by washing the needles three times with 15-milliliter batches of 95-percent ethanol. Final volume was adjusted to 50 milliliters. Formulation of the dye, extraction procedures, and interpretation of the results were the same as those developed by Neisess.[5/] The concentration of dye was determined by spectrophotoflurometric methods (Undefriend 1962, 1969) and the insecticide present was presumed proportional to the amount of dye detected. Guidelines for preventing photodecomposition of the dye followed those of Akesson and Yates (1963).

Residue Analysis and Efficacy Evaluation

From five trees in each treatment, one shoot was removed from the first whorl of lateral shoots at 2, 7, 14, and 21 days post-treatment and subsequently analyzed by GLC (Richmond and others 1979) to assess the translocation and persistance of acephate and its metabolite, metamidophos. At 30 days post-spray, the leaders were removed above the terminal whorl, bagged, tagged, and transported to the laboratory to assess insect mortality and determine insecticide distribution. Each terminal shoot was split longitudinally and examined for evidence of larval mining and dead or alive larvae. One half of the shoot was used for assessment of acephate and metabolites in four tissues: needles, epidermis-cortex-phloem (bark), xylem, and pith. The other half of the longitudinally split leader was used to assess vertical distribution of acephate. This half of the leader was cut into 1-centimeter lengths for analysis.

Harvested tissues were kept frozen in dry ice until the analysis was performed after the method of Richmond and others (1979).

Biosynthesis of Metabolites

Metabolic fate and percent transport from source-to-sink tissues was determined under greenhouse conditions, because percent distribution cannot be determined from the field data.

Four 2-year-old potted Jeffrey pines with rapidly growing new terminal shoots were selected. Acephate, ^{14}C-methylthiol labeled (52 µCi/µmole), was formulated in a 1:1 mixture of ethanol and water and 0.01 percent (wt/vol) of Vatsol OT to a concentration of 0.01 µCi/50 µℓ. ^{14}C-acephate (50 µℓ, 3.9 µM) was applied to the adaxial surface of several needles from the previous year's growth at the base of the terminal shoot using a 50 µℓ micropipette attached to a Model M ISCO microapplicator to dispense the volume slowly, so that several needles could be painted. Seven days after treatment the terminal shoot and the treated needles were excised and extracted as described by Richmond and others (1979) modified by use of 95 percent ethanol as the extraction solvent rather than ethyl acetate. The four replications were processed individually through the entire analytical procedure. Thin layer chromatography (TLC) and liquid scintillation counting techniques were similar to those described by Bull (1979). Metabolites were located on the TLC plates by autoradiography and identified by co-chromatography with known analytical standards.

RESULTS

Spectrophotofluorometric analysis of the spray deposits show that average acephate deposit on the treated shoots (source) was 9.76 ppm (n = 19, SD = 3.83), where ppm is µg acephate/g fresh weight of tissue. Some of the acephate was translocated via phloem tissue to the new terminal and lateral shoots (sinks) where the concentration accumulated over time (fig. 1). During the first two days post-spray the concentration reached 74 percent of the maximum value to be attained at 14-days post-spray. At 21-days the concentration was less than 1 ppm.

Total toxicant translocated from the previous year's growth was analyzed for distribution from the basipetal to acropetal end of the leader at 1-centimeter intervals (fig. 2). Only small amounts of acephate or methamidophos were detected, thus they were combined. The toxicants were evenly distributed over the length of the shoot, although minor concentration differences were found from one section to the next.

The distribution of estimates of acephate were concentrated variously in the tissue types found in the terminal shoot (table 1). During the time frame of this study, needles appear to be the major depository of imported acephate. The remaining acephate was equally distributed between bark and pith--10.3 percent

of available acephate was located in the bark, 3.8 percent was found in the pith.

If the concentration of acephate found in the sinks is greater than that found in the sources, then the estimated accumulation ratio should exceed a value of one. Table 1 summarizes the accumulation ratios for each tissue.

Methamidophos is biosynthesized from acephate in living plant tissue. It is normally found in plants treated with acephate in concentrations ranging from 5 to 10 percent of its acephate complement. Methamidophos concentrations and distribution were established for the same samples used to determine acephate concentration (table 2). Methamidophos accumulation in the pith and needles was similar to acephate accumulation--13.8 percent of the available methamidophos was phloem transported to the pith, 56.3 percent was imported into the needles. The accumulation ratios are also shown for metnamidophos in relationship to various sink tissues.

Studies were conducted with ^{14}C-acephate to determine mass transport and metabolic fate. The distribution of ^{14}C-acephate between photosynthate source and photosynthate sink is displayed as a percentage of the dose applied (table 3). Most of the acephate (56.9) remained at the site of application, some acephate (12.54) was found in the new growth. Thus, from the total pool of acephate available, only 16.18 percent was translocated. The remainder of the acephate was converted to methamidophos and three additional metabolites--O,O-dimethylphosphoramidothionate, O,O=dimethylphosphoramidate, and O,S-dimethylphosphorothiolate. Some ^{14}C was found in the ethanol insoluble residue, indicating that further unidentified metabolites also may be present.

The combined concentrations of acephate and metamidophos obtained in the pith of the shoots was not toxic to the larvae of *E. sonomana* (table 4). The data show that treated and untreated trees received an almost identical number of attacks. The presence of an emergence hole in a mined shoot was interpreted to mean that a larva successfully completed feeding and left the shoot for pupation. When larvae could not be found in a mined shoot and in the absence of an emergence hole, it was assumed that survival of larvae in terminal shoots on acephate treated trees was actually significantly higher (86 percent) than on the untreated trees (64 percent).

DISCUSSION

Acephate is the most phloem-mobile systemic insecticide commercially available at this time. Our results indicate that its phloem mobility in Jeffrey pine shoots is limited. Other researchers have reported similar findings with other plant species

(Bull 1979, Horler and others 1975, and Larson 1976).

Protecting the terminal shoot during the spray operation by bagging restricts the acropetal transport pathway to phloem tissue. Thus, the experiment is a test of the phloem transport characteristics of acephate and its metabolites. Both acephate and methamidophos accumulated in the needles against their own concentration gradients, that is, estimated concentration in the sink exceeded that in the old needles (source). The accumulation ratios for methamidophos were consistently greater than one for all tissue systems tested. We conclude that the molecular structure of methamidophos closely mimics that of a weak organic acid and some methamidophos is actively loaded into the phloem system.

The percent translocation of acephate can be estimated by comparing the acephate concentration in the source and sink columns of table 3, where it is computed from ^{14}C-acephate studies. Using potted trees under greenhouse conditions, we find the percent translocation for acephate to be 16 percent. Methamidophos was more phloem-mobile: an estimated 37 percent of the applied dose was translocated from source to sink.

Neither acephate nor its toxic metabolite, methamidophos, are persistent. Data (fig. 1) indicate that the residual half-life is about 18 days, but the terminals retain residues for at least 30 days (tables 1 and 2). Sundaram and others (1977) have reported low levels of acephate (3.25 ppm) in spruce trees (*Picea glauca*) after 21-days post-treatment. Methamidophos levels were reported to be 0.41 ppm. Others have found similar results: Werner 1974, Lindquist and Kreuger 1975, and Larson 1976. Rapid disappearance of acephate is associated with its volatile nature under field conditions (Horler and others 1975, Larson 1976) and its rapid metabolism to nontoxic polar metabolites (Bull 1979).

The efficacy data reported by Neisess and others (1976) and Brewer and O'Neal (1977) implies that acephate or its metabolite methamidophos has some persistence beyond 35 days post-spray in Douglas-fir. Larson (1976) attributes these low levels of persistence to methamidophos, which has been biosynthesized from acephate. In cotton, these levels can reach concentrations of 9.1 percent of the applied dose at 7 days post-treatment (Bull 1979).

Clearly, we were unsuccessful in killing the *Eucosma sonomana* larvae feeding in the pith of the growing pine shoots. The feeding LC_{50} values of acephate and methamidophos for *E. sonomana* larvae are unknown. The pith of the shoots contained an average 1.01 ppm of acephate and 0.43 ppm methamidophos at 30 days post-spray. On the basis of our data (fig. 1), we estimate the peak concentration of toxicants reached at 14 days post-spray was between 4.9 and 7.0 ppm. A lethal dose of toxicant for *Eucosma*

sonomana larvae evidently is considerably higher.

Accumulation of a dose of these insecticides at LC_{90} or greater in the pith may be an impossibility, since the major photosynthate sink appears to be the needles. The parenchyma cells that make up the pith tissue are differentiated early in the development of the shoot. The pith is partially destroyed during growth of the stem. Thus, the pith is not a sink after shoot elongation starts. In order for phleom-mobile insecticides to accumulate in the pith, applications would have to be made at least one or perhaps two weeks before shoot elongation commences, at a time when the pith parenchyma cells are differentiating and are photosynthate sinks. This strategy may result in higher concentrations in the pith soon after application, but metabolic degradation during the metabolic degradation during the post-spray period before the larvae were exposed to the pith tissues may largely cancel the gains made by treating at an earlier date.

Concentration of the majority of translocated toxicant in the new needles suggests that acephate would be an excellent candidate for testing against insect larvae that feed upon or within pine needles. In fact, Brown and others (1979) have shown this to be true in the case of the Jeffrey pine needle miner (*Coleotechnites near* milleri *[Busk]*).

CONCLUSIONS

Acephate insecticide has been shown to exhibit phloem-mobility in Jeffrey pine. It was translocated from old growth foliage to new terminal and lateral shoots. Highest concentrations were found in the needles of the growing leader. Acephate and its toxic metabolite, methamidophos, persisted in the leader for more than 30 days, but a half-life of 18 days was found. Acephate concentrations found in the pith were not toxic to *Eucosma sonomana* larvae.

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FOOTNOTES

1/ We thank Dr. M. Look for the synthesis and purification of ^{14}C-acephate and Linda Miller, Christopher Fridia, Peggy Chung, A.B.Y. Tang, and Lydia Maruszko for their assistance in the chemical analysis.

2/ Trade names and commercial products or enterprises are mentioned solely for information. No endorsement by the U.S. Department of Agriculture is implied.

3/ This publication reports research involving pesticides. It does not contain recommendations for their use, nor does it imply that the uses discussed here have been registered. All uses of pesticides must be registered by appropriate State and/or Federal agencies before they can be recommended.

4/ K. J. Stoszek. 1973. Bionomics of the western pine shoot borer *Eucosma sonomana* (Lepidoptera: Tortricidae) in southeastern Oregon. Unpublished manuscript. On file at Pacific Southwest Forest and Range Experiment Station, U.S.D.A. Forest Service, Berkeley, Calif.

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KEY WORDS

Orthene; *Pinus jeffreyi* L.; methamidophos; phloem; pine shoot borer; translocation.

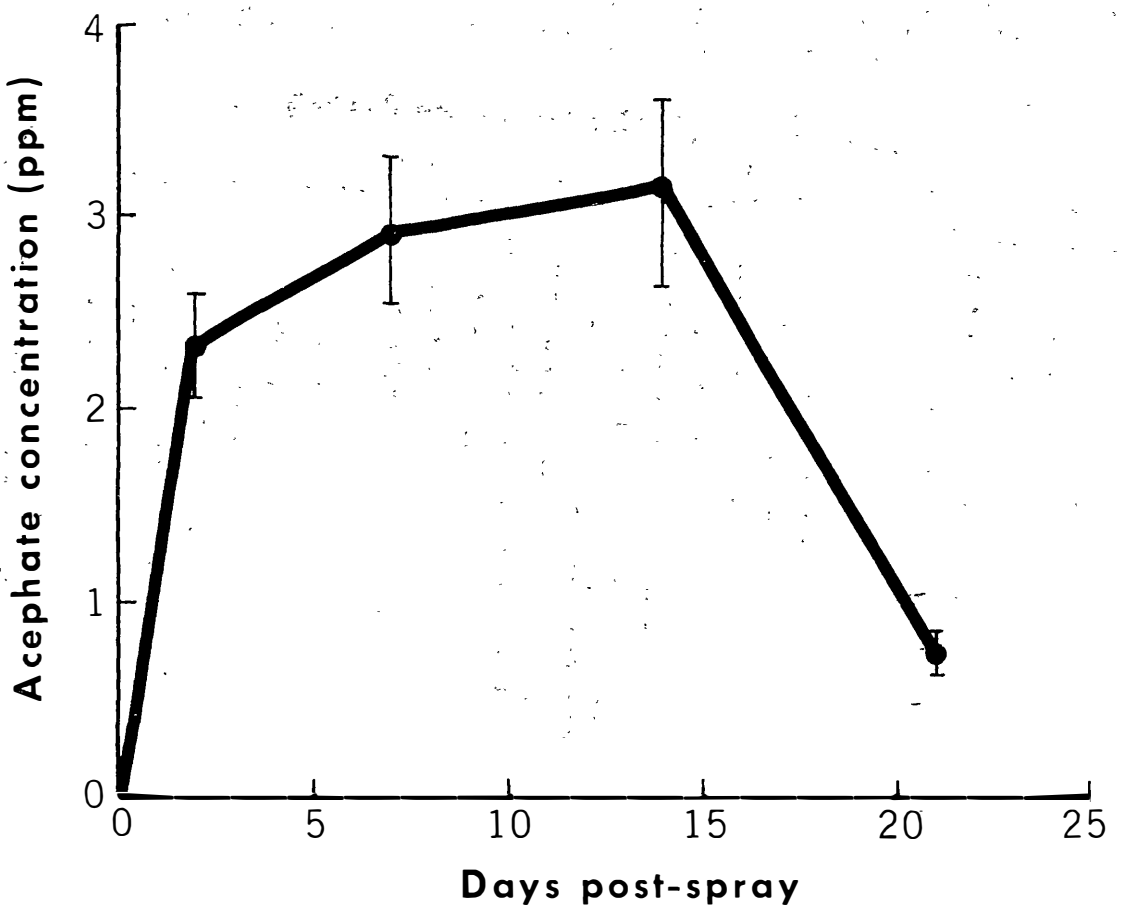

Fig. 1. Acephate concentrations in ppm in the lateral shoots of the first whorl below the terminal shoot of 15-year-old Jeffrey pine trees over time. Each data point is the mean of five replications. The vertical line extends ± 1 SD from the mean.

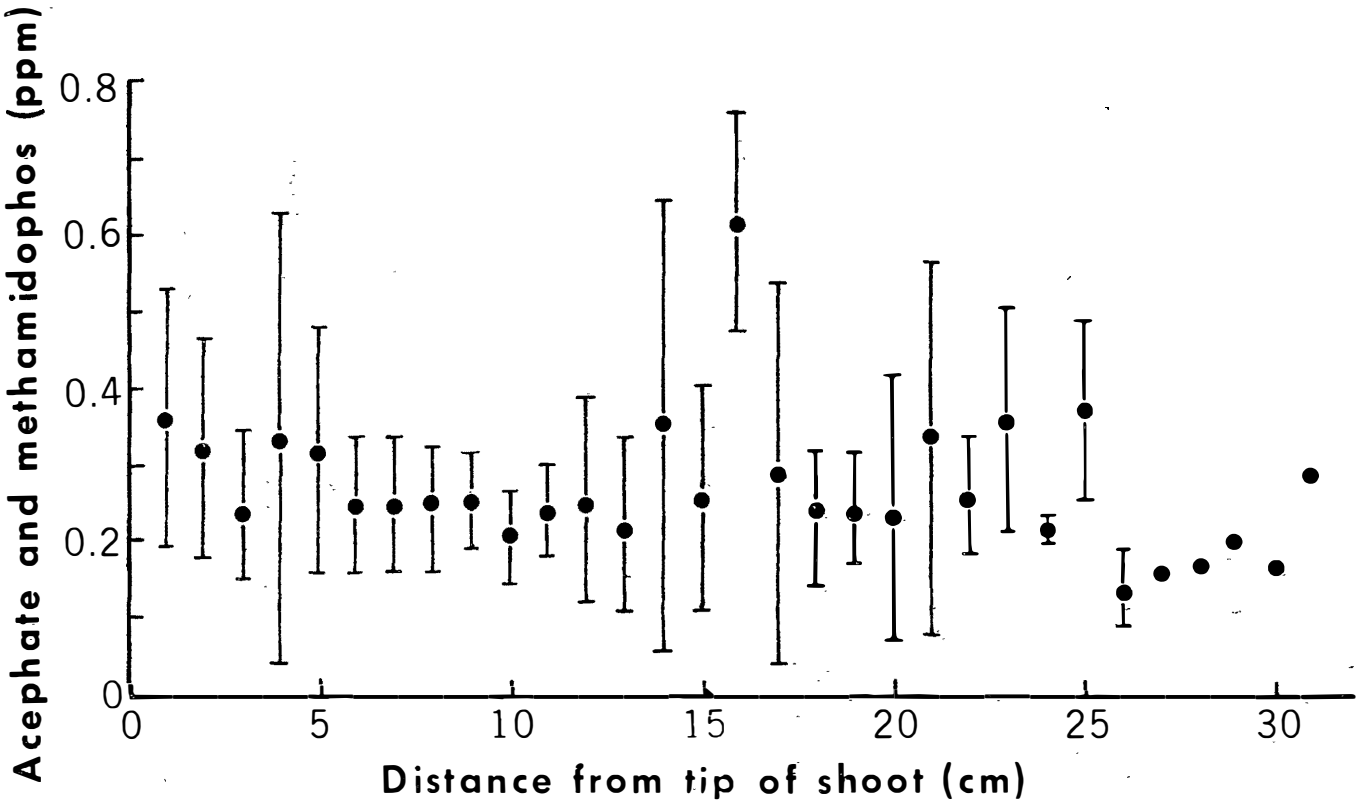

Fig. 2. Methamidophos and acephate concentrations found in the terminal shoot of Jeffrey pine at 30 days post-spray after phloem transport as a function of distance from the photosynthate source.

Table 1. Acephate (Orthene^{R}) concentration in the various tissues of the terminal shoot of Jeffrey pine (*Pinus Jeffreyi* L.) at 30-days post-spray after phloem transport and in the treated old growth.[1/]

Tissue	Samples	Concentration (ppm)	SD	Total Acephate (percent)	Accumulation Ratio[2/]
Treated Old Growth (Source)	19	5.57	2.08	--	--
Terminal Shoot[3/] (Sink)	50	3.00	8.90		
Needles	48	7.96	4.26	85.6	1.43
Bark	50	0.96	6.26	10.3	0.17
Xylem	51	0.05	0.20	0.3	0.01
Pith	49	1.01	2.11	3.8	0.18

1/ Terminal shoot was protected during application of acephate. Residues observed are due to systemic effects only and were determined by GLC.

2/ Accumulation ratio equals the concentration in sink divided by the concentration in the source, that is, accumulation ratio for needles equals 7.96 ÷ 5.57 = 1.43.

3/ Total acephate concentration in all tissues was 2.336 mg.

Table 2. Concentration of methamidophos as a metabolite found in various tissues of the terminal shoot of Jeffrey pine (*Pinus Jeffreyi* L.) at 30-days post-spray after phloem transport and in the treated old growth.[1/]

Tissue	Samples	Concentration (ppm)	SD	Total Methamidophos (percent)	Accumulation Ratio
Treated Old Growth (Source)	19	0.11	0.31	--	--
Terminal Shoot[2/]	50	0.27	0.69		
(Sink)					
Needles	48	0.54	1.34	56.3	4.91
Bark	50	0.14	0.13	13.8	1.27
Xylem	51	0.27	0.33	16.1	2.45
Pith	49	0.43	0.55	13.8	3.91

1/ Terminal shoot was protected during application of methamidophos. Residues observed are due to systemic effects only and were determined by GLC.

2/ Total methamidophos concentration in all tissues was 213 µg.

Table 3. Percent distribution of ^{14}C-acephate and its ^{14}C-metabolites between the photosynthate source and the photosynthate sink as a function of the concentration of ^{14}C-acephate applied to Jeffrey pine needles. Values are means of four replicates.

Fraction Name	Source		Sink		Translocation (percent)	
	Mean	SD	Mean	SD	Mean	SD
Ethanol Soluble:						
N(acetyl) O,S-dimethylphosphoramidothionate (acephate)	56.96	21.34	12.54	9.54	16.18	11.74
O,O-dimethylphosphoramidothioate	0.99	0.81	0.86	0.57	49.33	30.47
O,S-dimethylphosphoramidothioate (methamidophos)	0.87	0.70	0.46	0.41	36.95	22.92
O,O-dimethylphosphoramidate	0.26	0.10	0.17	0.08	39.81	19.91
O,S-dimethylphosphorothiolate	0.40	0.16	0.25	0.14	41.24	22.87
Ethanol Insoluble	22.11	29.80	4.23	5.75	25.35	35.39
Sum of Source and Sum of Sink	81.56	22.37	18.39	12.91		
Sum of Source plus Sum of Sink	99.94	10.30				

Table 4. Numbers of *Eucosma somomana* larvae found in Jeffrey pine terminal shoots at 30 days post-treatment in check trees and trees treated with acephate.

Treatment Type	Larvae per 100 Shoots				Total Attack per 100 Shoots[1/]
	Live	Emerged	Dead	Missing[2/]	
Check	18	6	5	7	36
Acephate	26	6	3	2	37

1/ No significant difference by chi-square.

2/ Indications of larval mining but no evidence of live or dead larvae or of exit hole.